

CLAIMS:

1. A new method for the conjugation of chorin *e6* to transferrin by first immobilizing
transferrin to an anion exchange gel, as described in the summary of the invention:

5 Synthesis of Chorin e6-transferrin. Said gel is, but is not limited to, quaternary
aminoethyl-sepharose (hereafter referred to as QAE sepharose); all solid supports
such as polystyrene, cellulose, etc., containing quaternary amine or positively charged
functional groups can be used for the preparation of chorin e6-transferrin.

10 2. The claim of 1 where the immobilized transferrin is reacted with chlorin *e6* in the
presence of, but not limited to, 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide
hydrochloride (hereafter referred to as EDC), in the presence of a detergent, and the
synthesized conjugate released using high salt. The coupling agent is, but is not
limited to EDC. Other commonly used compounds such as cyclohexyl-3(2-
15 morpholinoethyl) carbodiimide can serve the same function.

20 3. The claim of 1 and 2, where the presence of a detergent is required for optimum
formation of and release of the conjugate from the gel. The detergent is, but not
limited to 3-[(3-cholamidopropyl) dimethylammonio]- 1-propanesulfonate
(hereafter referred to as CHAPS). Other detergents, such as octyl glucoside, Triton
X-100, Tween20, etc. can serve the same function.

4. Preparation of transferrin-QAE sepharose. The claim of 1,2, and 3, wherein iron-free
or iron saturated transferrin from any species is immobilized or bound to, but not
limited to, quaternary aminoethyl-sepharose while in a solvent of, but not limited to,
20 mM phosphate buffer, pH 7.4 (20 mM Na₂HPO₄, adjusted to pH 7.4 with
5 KH₂PO₄; hereafter referred to as PB) containing a detergent of, but not limited to,
CHAPS, at a concentration of, but not limited to, 2 mM (solvent hereafter referred to
as PB/CHAPS); and the gel is washed free of unbound transferrin in like solvent,
after binding occurs to saturation and completion.

100-223-0112

10 5. Preparation of chlorin e6-transferrin-QAE sepharose. The claim of 1, 2, 3, and 4 where
4, but not limited to 4, volumes of chlorin e6 in, but not limited to, PB/CHAPS is
added to 1, but not limited to 1, volume of washed transferrin-QAE sepharose, and to
this is added 0.25, but not limited to 0.25, volumes of EDC in a solvent of, but not
limited to, purified water; and this mixture is incubated for, but not limited to, 20
15 minutes, at, but not limited to, room temperature, all while mixing, or by the use or
any methodology, to ensure a uniform reaction which proceeds to saturation and
completion.

6. Preparation of chlorin e6-transferrin-QAE sepharose, alternate to aim 5. The claims of
20 1, 2, 3, and 4, where chlorin e6 at, but not limited to, 1 mg/ml, dissolved in, but not
limited to, PB/CHAPS is combined with EDC at, but not limited to, 1 mg/ml (initially
dissolved at, but not limited to, 10 mg/ml in, but not limited to, water), for, but not
limited to, 20 minutes, at, but not limited to, room temperature, and subsequently

exposed to an excess of QAE-sepharose in, but not limited to, PB/CHAPS for, but not limited to, 20 minutes, at, but not limited to, room temperature; wherein the desired modified chlorin e6 remains unbound to and is separated from the gel by, but not limited to, centrifugation. Where 4, but not limited to 4, volumes of this modified chlorin *e6*, is added to 1, but not limited to 1, volume of washed transferrin-QAE sepharose, and this mixture is incubated for, but not limited to, 20 minutes, at, but not limited to, room temperature, all while mixing, or by the use or any methodology, to ensure a uniform reaction which proceeds to saturation and completion.

10 7. The claim of 5 and 6 wherein the chlorin e6-transferrin-QAE-sepharose and other
insoluble material is washed of free chlorin e6, modified chlorin e6, and other soluble
material by, but not limited to, repeated centrifugation from and re-suspension in a
solvent of, but not limited to, the PB/CHAPS solvent of claim 5.

15 8. The claim of 7 wherein the formed chlorin e6-transferrin is released from QAE
sepharose by exposure to, but not limited to, PB/CHAPS containing, but not limited
to, 0.5 M NaCl.

9
10. The claim of 8 wherein the released chlorin e6-transferrin is freed of the high salt
20 buffer or placed in a new solvent system by, but not limited to, dialysis. The claim
whereby other methodologies such as, but not limited to, gel filtration or
ultrafiltration, are used to eliminate the salt from the chlorin e6-transferrin.

11. The claim of 10 whereby chlorin e6-transferrin is further purified by being placed in a
low pH solvent of, but not limited to 25 mM sodium acetate, pH 4.8, and is reacted
with a negatively charged matrix such as, but not limited to, sulfo-propyl sepharose,
in a solvent of, but not limited to 25 mM sodium acetate, pH 4.8; whereby the chlorin
e6-transferrin binds to the matrix and any free, un-modified chlorin e6 does not.

12. The claim of 11 whereby chlorin e6-transferrin immobilized to sulfo-propyl
sepharose is washed free of soluble material by, but not limited to, repeated
centrifugation from and re-suspension in a solvent of, but not limited to, 25 mM
sodium acetate, pH 4.8.

13. The claim of 10, 11, and 12 where the sulfo-propyl sepharose bound chlorin e6-
transferrin is released by, but not limited to, PB/CHAPS containing, but not limited
to, 1.0 M NaCl, and is placed in a new solvent by, but not limited to, dialysis.

14. The claim of 1, 10, and 13, where said transferrin-chlorin e6 conjugate is added to
cells in culture. The cells are, but not limited to, tumor cells. The tumor cells are,
but not limited to, breast cancers, melanoma, etc., and all other cells or tumor cells
possessing substantial amount of functional transferrin receptors or other factors
causing transferrin binding to, association with, or internalization into the cells.

15. The claim of 14 where said cultured tumor cells or other cells associated with chorin
e6-transferrin are damaged or destroyed by exposure to light.

16. The claim of 1, 10, and 13, where said chlorin e6-transferrin conjugate is delivered
into tumor bearing humans or animals by, but not limited to, injection, or other
methods such as, but not limited to, catheter, etc.

5

17. The claim of 16 where said chorin e6-transferrin-tumor cells residing in said humans
or animals are damaged or destroyed by exposure to light, where said light is any
light source capable of converting chlorin e6 to the toxic form, including, but not
limited to, fluorescent, incandescent, and laser light.

10

18. The claim of 1, 10, 13, 16, and 17 where said transferrin is purified from, but not
limited to, the blood, serum, or plasma of a cancer patient or animal, is then
conjugated with chlorin e6, delivered into that patient or animal, and that patient's or
animal's tumor(s) is irradiated by light.

15

19. The claim of 17 and 18 where tumor cells in the treated patient or animal are
damaged or destroyed directly by the chorin e6-transferrin/light therapy, or indirectly
from subsequent destruction of light-damaged tumor cells by other events such as, but
not limited to, recognition and destruction of light-damaged tumor cells by the
immune system, and the patient's or animal's prognosis is improved

20

19

20. The claim of 16, 17, 18, and 19 where circulating chlorin e6-transferrin-tumor cells
are destroyed by passage of the patient's blood through a light-irradiation instrument
positioned outside the body.

20

21. The claims of 16, and 17, where transferrin-binding, associating, or internalizing
cells other than tumor cells are selectively destroyed using these methods, in the
treatment of other conditions or diseases.

RE 1/26

21

22. The claims of 16 and 17 where treatment of cancer-bearing humans or animals by
10 administration of chlorin e6-transferrin followed by light exposure is used as an
adjunct treatment for cancer, or any other condition, alongside existing conventional
or other treatments.

22

23. The claims of 16 and 17 wherein said treatment of humans or animals by
15 administration of chlorin e6-transferrin followed by light exposure is repeated
multiple times to eliminate disease or for other purposes.

23

24. The claims of 1, 14, 15, 16, and 17, wherein said treatment of cultured cells or
humans or animals by administration of chlorin e6-transferrin followed by light
20 exposure is used for any diagnostic or research purposes.

Synthesis, and photodynamic therapy-mediated anti-cancer, and other uses of
chlorin e6-transferrin. Philip Cavanaugh, Inventor

26
25. The claim of 1, 10, 13, 16, and 17, wherein said transferrin is likewise conjugated
with chlorin e6 and utilized in any way, whether activated to the toxin form or not, or
activated to the toxin form in any way, by any methodology.

5

100-000-0000-0000